

**Remarks:**

**Claim Status**

Claims 40, 42-47, 49-54, and 56-60 are currently pending.

Claims 40, 42-47, 49-54, and 56-60 have been rejected.

Applicant respectfully traverses the rejection of claims 40, 42-46, 49-52, and 60 in view of the arguments and amendments herein. Claims 41, 47-48, and 53-59 are cancelled.

A terminal disclaimer was filed and accepted to remove one the double patenting objection over USPN 6,989,235.

**Rejections under §103**

**1. Prima Facie Rejection Has Not Been Made**

Claims 40, 42-45, 47 and 49-52 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Yasuda et al (Nature 2001) in view of Sonnichsen (Physical Review Letter Pub 112002) (as evidenced by Mock (Nano Letters Pub 412002)) and in further view of Pettingell et al (US Patent 6449088 Filed 1993). Claims 47, and 56-59 have been cancelled. Clarifying amendments have been made to the remaining claims and applicant submits that these are allowable over the references whether taken singly or in combination. Claim 40 has been amended and is allowable over the references as amended and in view of the arguments presented hereinbelow.

Claims 54 and 56-59 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yasuda et al (Nature 2001) in view of Sonnichsen (Physical Review Letter Pub 112002) (as evidenced by Mock (Nano Letters Pub 412002)). Claims 54 and 56-59 have been cancelled.

Claims 46 and 53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yasuda et al (Nature 2001) in view of Sonnichsen (Physical Review Letter Pub 112002) (as evidenced by Mock (Nano Letters Pub 412002)), and Pettingell et al (US Patent 6449088 Filed 1993) as applied to claims 40 and 47 above and in further view of Felder (US Patent 6232066). Claim 46 has been amended to traverse the rejection. Claim 53 has been canceled.

Claim 40 has been amended to add the limitation "wherein the detection of a target molecule is enabled when the target molecule forms a specific structural link between the molecular motor and the rod-shaped nanoparticle such that the molecular motor causes the rod-shaped nanoparticle to rotate." At least this limitation of claim 40 is not found in any permissible combination of the references. Thus claim 40 is allowable over the references as are dependent claims 42-46 and 49-52 including the limitations of claim 40.

Yasuda is cited for disclosing attaching a nanobead to an F1-ATPase motor, rotation using a nanobead and imaging with laser. However, Yasuda shows bonding to a rotating arm with biotin and streptavidin and no target matching DNA linkage is shown.

Sonnichsen is cited for disclosing gold nanorods. As evidenced by Mock a property of a nanorod is to alternately produce red and green polarized light when illuminated with polarized light along the axes. No target matching DNA linkage is shown in Sonnichsen or Mock.

Pettingell et al. discloses using polarizing microscopes which use polarizers.

Felder is cited for disclosing oligonucleotide links as an anchor. No target matching DNA linkage between a rotating molecular motor and a nanoparticle suitable for causing the nanoparticle to rotate is discussed.

Combination of Yasuda and Felder is not permitted under §103 because the references teach away from each other and the combination destroys the intended functions of the references. Turning first to Felder, Felder teaches detection of target molecules by using probes for detection of targets. The probes are defined as: "... a substance, e.g. a molecule, that can be specifically recognized by a particular target." (See, Felder, Col. 4. ll. 34-35) Felder does not use or need rotation to detect targets, nor F<sub>1</sub>-ATPase enzymes to produce rotation.

In contrast, Yasuda teaches the use of gold beads to detect rotation in F<sub>1</sub>-ATPase enzymes. The gold beads produce reflections that indicate rotation.

The references are not properly combined because it is not suggested by the references how to combine Yasuda and Felder to achieve the limitations of the present

invention without destroying the intended functions of the references. More specifically, the references are not properly combined because it is not suggested by the references how to substitute the rotating beads of Yasuda for the Felder probes while following the teachings of the references.

For example, since Felder does not disclose using rotation to detect targets, combining Felder with Yasuda would destroy the intended purpose of Yasuda which is to detect  $F_1$ -ATPase rotation and not non-rotating targets as in Felder. Similarly, there is no suggestion in either of the references for using Yasuda's rotational system combined with Felder to detect targets since Yasuda does not teach target detection by light scattered through  $F_1$ -ATPase rotation of gold beads and neither does Felder.

While the combination has been asserted applicant respectfully submits that it has not been shown how the combined references teach the step of disposing a detection DNA strand between the nanoparticle and the molecular structure in a detection system where targets induce rotation of an  $F_1$ -ATPase molecule. For example, if the structure of Yasuda were substituted for Felder's structure, as shown, for example, in FIG. 1, the "Detection 1" probe would have no useful function, thus destroying Felder's intended technique. This does not even address the fact that Yasuda's structure would also be significantly altered by adding elements from Felder between Yasuda's  $F_1$ -ATPase enzyme and his gold bead. Further still, Felder specifies that his detection probe be "specific for a target of interest" (See, for example, Abstract). Even if it were allowable, hypothetically, to extend the argument to add Sonnichsen's gold particles to the combination, this addition would not solve the improper combination of Yasuda and Felder. Whereas the gold beads of Yasuda or (if allowed to be substituted) gold nanorods are not specific to "a target of interest," but only provide an indication of rotation. Thus, Yasuda and Felder teach away from each other and following their teachings would not lead one skilled in the art to the method of the present invention. Thus claim 40 is allowable over the references as are dependent claims 42-46 and 49-52 including the limitations of claim 40.

Claim 60, as amended, is allowable over the references

Claim 60 has been rejected under 35 U.S.C. 103(a) as being unpatentable over Yasuda et al (Nature 2001) in view of Sonnichsen (Physical Review Letter Pub 112002) (as evidenced by Mock (Nano Letters Pub 412002)), Felder (US Patent 6232066), and Greenberg (US Patent 53051 39). Applicant respectfully submits that claim 60 is allowable over the references in view of the arguments presented herein.

As argued above with respect to claim 40, claim 60 is non-obvious over the references due to the surprising results achieved with nanorods used in observing molecular rotation. For a claim to be found obvious in view of the references, all of the claim elements must be found in a properly combined set of references taken as a whole. Here the references do not disclose all of the claimed elements of the invention as set out in amended claim 60.

Further still, as argued above, the references fail to disclose at least the element of disposing a detection DNA strand between the nanoparticle and the molecular structure, wherein the detection DNA strand hybridizes with a target DNA strand such that if the target DNA strand matches the detection DNA strand they form a structural link between the molecular structure and the nanoparticle causing the nanoparticle to rotate... indicating detection of the target DNA strand.

Yasuda is cited for disclosing attaching a nanobead to an F1 ATPase motor, rotation using a nanobead and imaging with laser. However, Yasuda shows bonding to a rotating arm with biotin and streptavidin and no target matching DNA linkage is shown.

Sonnichsen is cited for disclosing gold nanorods. As evidenced by Mock a property of a nanorod is to alternately produce red and green polarized light when illuminated with polarized light along the axes. No target matching DNA linkage is shown in Sonnichsen or Mock.

As stated above, Felder is cited for disclosing oligonucleotide links as an anchor. No target matching DNA linkage between a rotating molecular motor and a nanoparticle suitable for causing the nanoparticle to rotate is discussed.

Greenberg discloses a polarizing filter and does not otherwise supply claimed elements missing in the disclosures of the other references.

Analogous to the argument above for claim 40, the Yasuda and Felder references in combination teach away from each other and the invention because none of the references, whether taken singly or in any combination, teach the claimed feature that a structural link suitable for rotating a nanoparticle linked to a molecular motor is created by the hybridization of a detection strand and a DNA target strand causing rotation which indicates detection of the target DNA strand. Thus, for this reason alone claim 60 is allowable over the references whether taken singly or in any combination.

2. Even If the References Are Properly Combined, Evidence Of Surprising And Unexpected Results Traverses Rejections Under §103

Unexpected results are a key factor showing non-obviousness as stated by the Supreme Court in the seminal case of *Graham v. John Deere Co.* Unexpected Results show inventiveness if results are surprisingly better or superior by a person of ordinary skill in the art, are not predictable nor obvious and the standard for comparison is closest prior art. Thus, even if, *arguendo*, the references are properly combined, the claims are not obvious and allowable over the references.

Evidence of non-obviousness is presented in the Declaration of Dr. Wayne Frasch submitted herewith. Dr. Frasch, an inventor here is skilled in the art as shown by his background and experience in nanotechnology. His statement references work published by York et al., entitled "Abundance of *Escherichia coli* F1-ATPase molecules observed to rotate via single-molecule microscopy with gold nanorod probes," *J Bioenerg Biomembr* (2007) 39:435–439 (hereafter referred to as "the Journal article," attached hereto as Exhibit A) .

He is a co-author of the Journal article and conceived of, and directed the experiments that were conducted, interpreted the results and wrote the majority of the text. As evidenced by the data reported in the Journal article, the method as claimed in claim 1 of the subject application exhibits surprisingly better and superior results that are not expected nor obvious as compared to the prior art.

As stated in the Journal article, the fraction of molecules observed to rotate for F1 was at best 10 % using other approaches including the use of **nanospheres** as rotation probes that were used by Yasuda et al. 2001. Because such a small percentage of the population of molecules has been observed to rotate, it has been difficult to conclude that the measurements made that characterize rotation are representative of the enzyme population as a whole. More importantly, the low abundance of rotating F1 molecules that can be observed using these other approaches including gold nanospheres as reported by Yasuda, makes it impractical if not impossible to adapt this as a means of detection of target molecules including DNA.

The fraction of rotating molecules observed when a nanorod was used instead of a nanosphere was found to be **5 to 30-fold higher** than reported previously with other methods, including the methods proposed by Yasuda et al., such that the majority of molecules can be observed to rotate. These data indicate that, in a surprising advance over the prior art, rotational measurements made using gold nanorods provide information of the F1-ATPase mechanism that is representative of the characteristics of the enzyme population as a whole. Thus, the detection of target molecules by rotation as evidenced by the rotation of gold nanorods becomes a practical and useful method for the first time using the claimed method of the present invention.

One skilled in the art following Yasuda and the other references would not predict or expect such a surprising and unexpectedly successful result. There is no teaching or suggestion in the references that using nanorods instead of nanospheres would result in up to a 30 fold gain (3000%) in sensitivity. Therefore, claim 40, claim 60 and all of the remaining pending claims including limitations from those claims are non-obvious over the references whether taken singly or in any combination and are allowable over those references.

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For these reasons, and in view of the above amendments, this application is now considered to be in condition for allowance and such action is earnestly solicited.

Respectfully Submitted,

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Date

/George A. Leone, Reg. No. 30567/  
George A. Leone  
Attorney/Agent for Applicant(s)  
Reg. No. 30567  
Citadel Patent Law  
9124 Gravelly Lake Drive SW  
Suite 102  
Lakewood, WA 98499  
Tel. 253-682-0246